



RESEARCH PROGRESS REPORT SUMMARY

Grant 02528: Developing a Next Generation Sequencing Diagnostic Platform for Tick-Borne Diseases

Principal Investigator: Pedro Diniz, DVM, PhD
Research Institution: Western University of Health Sciences
Grant Amount: \$120,983.00
Start Date: 6/1/2018 **End Date:** 11/30/2020
Progress Report: End-Year 1
Report Due: 5/31/2019 **Report Received:** 5/30/2019

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Original Project Description:

Diagnostic tests based on the detection of DNA from harmful organisms in clinical samples have revolutionized veterinary medicine in the last decades. Currently, diagnostic panels for several vector-borne organisms are available through universities and private labs in the USA and abroad. However, the vast majority of results from sick dogs are negative, which frustrates veterinarians and dog owners trying to reach a definitive diagnosis. These panels are based on the detection of previously known DNA sequences of each pathogen, which limits their ability to detect novel organisms. In this study, the investigators will adapt high-throughput next-generation sequencing (NGS) to the detection of tick-borne bacteria in dog blood in an effort to overcome the limitations of current diagnostics for tick-borne diseases. If successful, increasing the capabilities of NGS to detect infected dogs and to accurately determine which bacteria are responsible for disease will support the development of a better diagnostic tool to simultaneously advance canine and human health. This work expands on Dr. Diniz's previous AKC CHF-funded study #02292.

Publications:

Manuscript(s) in preparation:

- Persico E., Quorollo B., Thomas B., Hegarty B., Breitschwerdt E., Diniz P.P.V.P. Molecular prevalence of selected canine vector-borne pathogens in the United States (2008-2015). Journal of Veterinary Internal Medicine.

Manuscript(s) under final stages of preparation:



- Vasconcelos E.J.R., Geiger J., Oakley B., Diniz P.P.V.P. Best practices for applying Next-Generation Sequencing to vector-borne disease diagnostics. BMC Veterinary Research.
- Ong K., Vasconcelos E.J.R, Oakley B., Diniz P.P.V.P. Comparison of 16S rRNA variable regions for the identification of canine vector-borne pathogens. Vector-Borne and Zoonotic Diseases Journal.

Presentations:

- Vasconcelos E.J.R., Oakley B.B., Diniz P.P. Utilizing Omics Approaches to Better Understand and Diagnose Vector-Borne Pathogens. Lecture for undergrad students in Biological Sciences at the Harvey Mudd College, Claremont, CA. Invited by Dr. Eliot Bush (Associate Professor at HMC), Oct 3rd, 2018.
- Persico E., Qurollo B., Thomas B., Hegarty B., Breitschwerdt E., Diniz P.P.V.P. Molecular prevalence of selected canine vector-borne pathogens in the United States (2008-2015).
 - o Poster presentation at:
 - International Conference on Emerging Infectious Diseases (ICEID) August 27th, 2018 Atlanta, GA (Omni Hotel at CNN Center)
 - The 15th International Conference on Lyme Borreliosis and other tick-borne diseases (ICLB) September 13th, 2018 Atlanta, GA (Emory University/CDC campus)
 - o Oral Presentation at the American Public Health Association Annual Meeting (APHA) November 12th, 2018 San Diego, CA (San Diego Convention Center)

Report to Grant Sponsor from Investigator:

Diagnostic tests based on the detection of DNA from harmful organisms in clinical samples have revolutionized veterinary medicine in the last decades. Currently, diagnostic panels for several vector-borne diseases (VBDs) are available through universities and private labs in the USA and abroad. However, the vast majority of results from sick dogs are negative, which frustrates veterinarians and dog owners trying to reach a definitive diagnosis. These panels are based on the detection of previously known DNA sequences of each pathogen, which limits their ability to detect novel organisms. Using an innovative approach, our study proposes the adaptation of high-throughput next-generation sequencing (NGS) to the detection of tick-borne bacteria in dog blood to overcome the limitations of the current diagnostics. NGS is capable of generating millions of individual sequencing reads from each sample, allowing for the unbiased identification and characterization of multiple organisms from a single sample. With support from the AKC Canine Health Foundation, we are pioneering this strategy for the accurate detection of VBDs. With the advances in DNA sequencing of entire genomes of microorganisms, we are using cutting-edge techniques to identify regions of the genome that can be used for better detection and characterization of vector-borne organisms. In parallel, we continue to test the MolYsis™ technology, which has demonstrated to reduce host DNA concentration in infected dog blood samples confirmed by quantitative PCR assay. The AKC-CHF support has been instrumental for the development of better diagnostic tools in veterinary medicine.